Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.24, 2016

The Mechanism of Action and Effect on the Cervix of the Seed of *Ricinus communis* Var. Minor (RICOM-1013-J) in Women Volunteers in Nigeria

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This research and publication was partly funded by the African Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria.

Abstract

The seeds of *Ricinus communis var minor* (RICOM-1013-J) have been reported to prevent conception for a year, when taken as single oral dose of 2.5-2.7 gm. This study was undertaken to investigate the possible hormonal changes mediating its activity and also evaluate any potential relationship with cervical intraepithelial neoplasia in women. Sixty women volunteers attending a traditional herbal clinic in Jos, were recruited for this study and divided into treatment and control groups of 30 each. The study showed that RICOM-1013-J protected women participants against conception for a duration of 12 months, while maintaining regular menstrual cycles. Nausea, vomiting, weight gain, raised blood pressure and menstrual abnormalities commonly experienced with conventional oral contraceptive were absent. Further findings revealed a statistically significant elevation in plasma levels of prolactin in women administered RICOM-1013-J compared to controls (p<0.05). However, there was no significant difference in FSH and LH plasma levels between both groups. In addition, the prevalence of cervical intraepithelial neoplasia between women who received RICOM-1013-J and control group was not statistically significant (p>0.05). It is possible therefore, that RICOM-1013-J may act in part through an oestrogen induced hyper- prolactinaemic hypogonadal mechanism. This study has provided the first insight of hormonal changes in human volunteers administered RICOM-1013-J as contraceptive agent.

Key words: Ricinus communis; antifertility; oestrogenic; ovary; hyperprolactinaemia; intraepithelial neoplasia;

1. Introduction

Anecdotes that the seeds of *Ricinus communis* var minor, (RICOM -1013-J) provided anticonceptive activity have been expressed for decades by the Rukuba speaking people in Bassa Local Government Area of Plateau State, north central Nigeria. Consequently, Okwuasaba *et al*. (1991) demonstrated that when administered as 3-4 seeds, RICOM-1013-J protected fertile female volunteers against conception for 12 months. Similar findings

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u *et al*. 2010). the women of Rukuba was informed in part, by the absence of troublesome side effects often associated with conventional family planning pills such as nausea, headache, weight gain or raised blood pressure. Chemical pathological and toxicological profiling of renal and liver functions in women volunteers revealed that RICOM-1013-J possessed "very high margin of safety" (Das *et al*. 2000). Phytochemical screening of *Ricinus communis* has revealed presence of steroidal compounds and alkaloids (Onwurili & Anakwe 2001; Sani & Sule 2007).

The mechanism of action of the anticonceptive effect of RICOM-1013-J is poorly understood and so far based on some histological and pharmacological studies in animal models. Okwuasaba *et al*. (1997a; 1997b) demonstrated oestrogenic activity of ether extract of the seed in immature ovariectomized rats and mice, and that pretreatment with the extract altered electrical activity of the uteri and fallopian tubes, precipitating a state of prolonged inertia. The authors further showed decreased uterine responsiveness to some standard smooth muscle agonists such as oxytocin, ergometrine, PGF2α and acetylcholine. In addition, they found histological changes in

the ovaries that included numerous vacuolation of corpora lutea (suggestive of anovulation) and a distorted uterine histoarchitecture. Similar histological changes have also been reported in mice (Hou *et al*. 2010) and testes of male albino rats (Raji *et al*. 2006). Furthermore, McNeil *et al*. (2003) have shown that RICOM-1013-J exerted antiovulatory effect in adult cyclic Sprague-Dawley rats. These authors proposed a disruption of the delicate oestrogen-progesterone balance, on the ovaries and uterus as possible mechanism of action. Whilst it may be argued that oestrogen content of the seed caused the reported tissue changes and subsequent contraceptive effect as also reported by Briggs (1976), the absence of oestrogen related side-effects often experienced with conventional pill, but rarely reported with RICOM-1013-J (Isichie *et al*. 2000) may imply an insignificant direct role of oestrogen.

It is also possible that the oestrogenic activity of RICOM-1013-J would have clinical implications for consumers of this phytodrug, because of the role of oestrogen in human cervical cancers. Cervical cancers are an important and leading cause of deaths attributable to cancers in women (Brake & Lambert 2005). Frequently, invasive cervical cancers are preceded by a continuum of pre-invasive abnormal cellular transformations of variable duration. This change is often limited to the epithelium and referred to as cervical intra-epithelial neoplasia (CIN). Several studies have reported increased risk of developing cervical dysplasia and cancer through the use of oestrogen containing drugs and hormonal contraceptives by women (Epstein 2003; Smith *et al*. 2003; McFarlane-Anderson *et al*. 2008). In particular, the International Agency for Research on Cancer has identified use of combined oral contraceptives as a definite cause of human cervical cancer (Appleby *et al*. 2007).

The present study was designed to provide more insight into the effect of RICOM-1013-J on the hormonal interplay along the hypophyseal-pituitary axis, and to evaluate its possible effect on the prevalence of cervical intraepithelial neoplasia in women volunteers.

2. Materials and Methods

2.1 Plant material

The seeds were collected in Jos, Plateau State in north central Nigeria in the months of January and February, 2014, and prepared as previously described (Okwuasaba *et al.* 1991) by the traditional herbal consultant to the Department of Pharmacology, University of Jos, Dr. O. Azija. The plant was taxonomically authenticated by S. W. H. Huseini of the Department of Botany, University of Jos. A voucher specimen was deposited at the herbarium of the Department of Pharmacognosy, University of Jos.

2.2 Selection of participants and hormone assay

Fifteen women volunteers of comparable socioeconomic status attending the Traditional Herbal Clinic of Dr. Azija were recruited for the study following informed oral consent. Ten women comprised treatment group and were administered with 2.5-2.7 gm of RICOM-1013-J as single dose and 5 women served as control group. It was ensured that all women were not pregnant, taking any orthodox contraceptive and hypertensive. Both groups were followed-up for one year and in the 9^{th} month, 10 mL of venous blood was collected from the ante-cubital fossa on day 21 of the menstrual cycle using a 10 mL syringe, under aseptic conditions. Each blood sample was emptied into a plain tube and allowed to clot, followed by centrifugation at 3000 rpm, for 10-15 minutes. The supernatant sera was then collected in cryovial bottles and stored at -21^0 C. The refrigerated sera were later thawed at room temperature for prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) analysis using the Syntron ELISA technique at the research laboratory of the Department of Obstetrics and Gynaecology, Jos University Teaching Hospital.

2.3 Cervical smear and cytological preparations

Thirty volunteers from the pool of women attending the Traditional Health Clinic of Dr. Azija for family planning services and have been on RICOM-1013-J for at least 2 years were recruited for this study, following informed oral consent. Each had a pelvic examination for ovarian masses and followed by taking of cervical smear. A similar number of volunteers matched for socio-demographic status, except for negative history of RICOM-1013-J ingestion and attending the same clinic, were also recruited and served as control.

The procedure for obtaining a cervical smear required that each subject lied on her back and a sterile bi-valve vaginal speculum lubricated with water only passed and the cervix exposed. An Aryes spatula was then rotated over the whole surface of the cervix ensuring that the squamo-columnar junction was scrapped. The spatula was then smeared over the middle part of a glass slide and immediately fixed in alcohol. The slides were subjected to

staining with Heamatoxylin and Eosin and examined under microscope (Papanicolau, 1942), at the Department of Obstetrics and Gynaecology, Jos University Teaching Hospital.

2.4 Statistical Analysis of Data

Statistical analysis of significance between groups was determined in terms of ANOVA and Chi square for the comparison of differences between group means and proportions.

3. RESULTS

3.1 Contraceptive Efficacy

It was found that the oral administration of 2.5-2.7 gm of RICOM-1013-J contraceptive protected women volunteers against conception for 12 months. Sexual frequency ranged from 2-3 times per week and menstrual flow remained within normal limits, as shown in Table 1. There was no evidence of side effects monitored during the study, which included nausea, vomiting, headache, dizziness, raised blood pressure and allergic symptoms.

3.2 Hormone Assay Determination

The findings showed that prolactin levels were normal in the control group, with statistically significant difference in the levels of prolactin between women who were on RICOM-1013-J relative to control women ($P<$ 0.05) as reflected in Tables 1 and 2. There was no significant difference in the levels of gonadotrophins (FSH and LH) between both groups ($P>$ 0.05).

3.3 Histology of the cervix (Papanicolaou smear)

Results of histological examinations of the cervix showed no increased prevalence of intra-cervical neoplasia among women administered RICOM-1013-J relative to the control group. It was observed that out of 30 women who received RICOM-1013-J, eight (8) women had positive pap smears, while a similar number of control women, seven (7) also had positive pap smears. These findings were not significantly different for both groups ($P>$ 0.05) as in Table 3.

4. Discussion

Previous studies have reported the contraceptive efficacy of RICOM-1013-J in women volunteers (Das *et al*. 2000; Isichei *et al*. 2000). The present study confirmed the efficacy and tolerability of RICOM-1013-J in women volunteers, and may explain the high acceptability of RICOM-1013-J as a means of birth control. It was observed that plasma prolactin was significantly high in women administered with RICOM-1013-J compared to control ($P<0.05$) and these women could be classified as hyperprolactinaemic.

Hyperprolactinaemia may be responsible for the contraceptive efficacy of RICOM-1013-J. Human prolactin hormone is secreted predominantly by the lactophore cells of anterior pituitary gland, under a unique permanent dopamine (D_2) inhibition. This regulation ensures low basal prolactin levels (<440mIU/L) in women of reproductive age, and any observed prolactin peaks are the result of de-inhibition. However, direct stimulation of prolactin secretion has been reported to occur in the presence of some drugs (Falorni & La Torre 2007; Green & Brown 1988; Wieck & Haddad 2002). Oestrogen is particularly known to cause hyperplasia of the lactophore cells of the anterior pituitary gland resulting in increased plasma prolactin (Luciano *et al*. 1985; Molitch 1999; Reyniak *et al*. 1980). Prolactin is known to inhibit gonadotrophin releasing hormone (GnRH) causing decrease fertility in women (Molitch 2005).

Since RICOM-1013-J caused an increase in prolactin in all the women participants administered with RICOM-1013-J, it is possible that the anticonceptive efficacy of RICOM-1013-J is partly due to the hyperprolactinaemia observed in these women. Low gonadotrophins in turn cause defective ovulation and infertility by effecting abnormal development of ovarian follicles and luteal phase lag (Evans *et al*. 1982). Furthermore, it may directly impair the endocrine function of ovarian follicles resulting in loss of oestrous cyclicity and anovulation (Sonigo *et al*. 2012). In this study, the mean gonadotrophin values were lower in women administered RICOM-1013-J relative to control, although they were not statistically significant ($P>0.05$). This pattern of endocrine disruption and accompanying histological changes have been demonstrated in earlier studies of the effect of *Ricinus communis var minor* on reproductive organs of rodents (Hou *et al*. 2010; Okwuasaba *et al*. 1997; Raji *et al*. 2006). It is therefore plausible that RICOM-1013-J may act in part to inhibit ovulation in women through

prolactin mediated gonadal dysfunction. This proposition agrees with Goncim *et al*. (2010) who reported inhibition of follicular development in women administered RICOM-1013-J.

Oestrogen induces proliferative response by its activity on epithelial cells of human reproductive organs and this effect is opposed in the luteal phase by progesterone (Sutherland *et al*. 1983; Ciocca & Fanelli 1997). Its role in human cervical neoplasia has also been reported, based on the observation that proloned, unopposed use of synthetic oestrogen containing oral pills increased the risk of human cervical cancer by 2 to 4 times (Moreno 2002). The study did not find any statistical difference in the prevalence of cervical intraepithelial neoplasia between women who received RICOM-1013-J and control women ($P > 0.05$).

The study concludes that the seeds of *Ricinus communis var minor* (RICOM-1013-J) when taken as single oral dose of 2.5-2.7 gm, possess significant reversible antifertility activity and well tolerated by women volunteers. We further posit that the reversible antifertility efficacy of RICOM-1013-J may partly be mediated through an oestrogen induced hyperprolactinaemic hypogonadal mechanism, and that the use of RICOM-1013-J may not be associated with increased incidence of cervical intra-epithelial neoplasia. Although, these studies are cross-sectional and involve small samples and therefore, should be interpreted with caution, the public health implications of the findings lie in the potential of this phytodrug to offer safer and cheaper alternative to current hormonal contraceptives.

Acknowledgement

We gratefully acknowledge the technical assistance of Dr. Zuachi, of the department of Pathology, University of Jos, who read the pap smears and Dr. Imade of the Department of Obstetrics and Gynaecology, University of Jos, for the hormone analysis. We also thank Dr. Azija for providing the seeds of RICOM-1013-J and permission to recruit participants from her traditional clinic.

IJU was involved in the study design, collected field data, analyzed the data and prepared the draft manuscript. EOE partook in data collection and review of the manuscript, while RTM participated in the research design and data collection. OKF conceived the study, coordinated and reviewed the manuscript.

Declaration of conflict of interest: The authors have declared that no competing interests exist.

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Table 1: Hormone profile of women administered RICOM-1013-J relative to control

S/N	Age (yrs)	RICOM-1013-J Usage(yrs)	Sexual Frequency /week	Duration of Menses (days)	Prolactin (mIU/L)	LH (IU/L)	FSH (IU/L)
Control Group							
1	20	-	2-3	3	76	2.6	3.4
2	30	-	3	3	170	11	6.6
3	24	-	3	3-4	210	1.5	4.1
4	28	-	2-3	3	400	0.7	0.9
5	28	-	2	2-3	105	2.2	4.8
6	28	-	3	4	68	0.7	2.2
Treatment Group							
1	22	1	3	3	700	6.0	6.2
2	23	1	2-3	4	500	2.0	2.88
3	28	1	2	3	2400	0.76	1.4
4	30	1	2-3	3-4	2400	1.5	5.2
5	26	2	2-3	3	500	0.98	2.1
6	30	2	2	2-3	700	1.5	3.2
7	36	2	2-3	3	640	0.48	0.86
8	24	3	3	3	640	0.48	4.7
9	27	3	3	4	2400	6.76	1.4
10	22	4	2-3	3	664	2.0	3.5

Markedly elevated levels of prolactin in women administered RICOM-1013-J, while control women have normal levels

Table 2: Mean plasma hormone concentrations of women administered RICOM-1013-J

Hormone	Mean plasma level Control group (Mean$\pm$ *sem*)	Mean plasma Level RICOM-1013-J treated (Mean$\pm$ *sem*)
Prolactin	171.5 ± 50.0	1154.4 ± 272.72 *
LH	1.47 ± 0.32	2.25 ± 0.71
FSH	3.67 ± 3.75	3.14 ± 3.04

* Statistical significance in the mean Prolactin values between women in treatment and control groups ($P < 0.01$). No statistical difference in mean levels of FSH and LH between treatment and control groups ($P > 0.05$)

Table 3: Prevalence of positive cervical smears

	CONTROL GROUP			STUDY GROUP		
Age (yrs)	Frequency	$\bar{x}$ use (yrs)	No of +ve smears	Frequency	$\bar{x}$ use (yrs)	No. of +ve smears
24-28	4	-	-	8	2	-
29-33	2	-	1	5	2.5	-
34-38	8	-	3	5	2.3	2
39-43	2	-	-	2	2	-
44-48	4	-	1	1	3	-
49-53	5	-	1	4	3	3
54-58	2	-	-	1	5	-
59-63	3	-	1	4	4	3
	30		7	30		8
Prevalence rate control = 23.33%					Prevalence rate study = 26.67%	

Pearson Chi-square = 4.152; Asymptotic significance = 0.386 No statistical difference between control and study groups (P> 0.05)